



UNIVERSITÀ DEGLI STUDI DI MILANO

*Department of Food, Environmental and Nutritional Sciences
Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente*

Shedding light on crystals and white spots in cheese

D'Incecco P.¹, Limbo S.¹, Faoro F.², Hogenboom J.¹, Rosi V.¹, Pellegrino L.¹

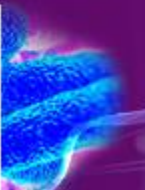
¹ Department of Food, Environmental and Nutritional Sciences
and

² Department of Agricultural and Environmental Sciences, University of Milan, Italy.

Oludeniz, 19-23 October 2015

InterM2015

3rd International Multidisciplinary
Microscopy and Microanalysis
Congress & Exhibition
Sentido Lykia Resort, Oludeniz, TURKEY
October 19-23, 2015



Case study: extra hard cheeses

Standard



... and this?



Extra hard cheeses: Background (I)

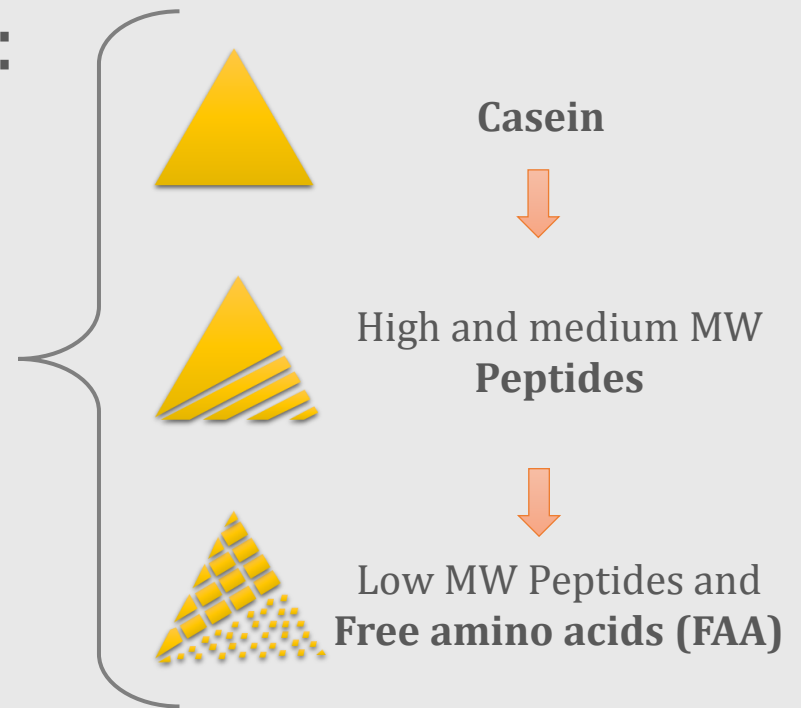
- Raw milk
- Natural whey starter
- Calf rennet
- Cutting and Cooking curd
- Long ripening (> 9 months)



Background (II)

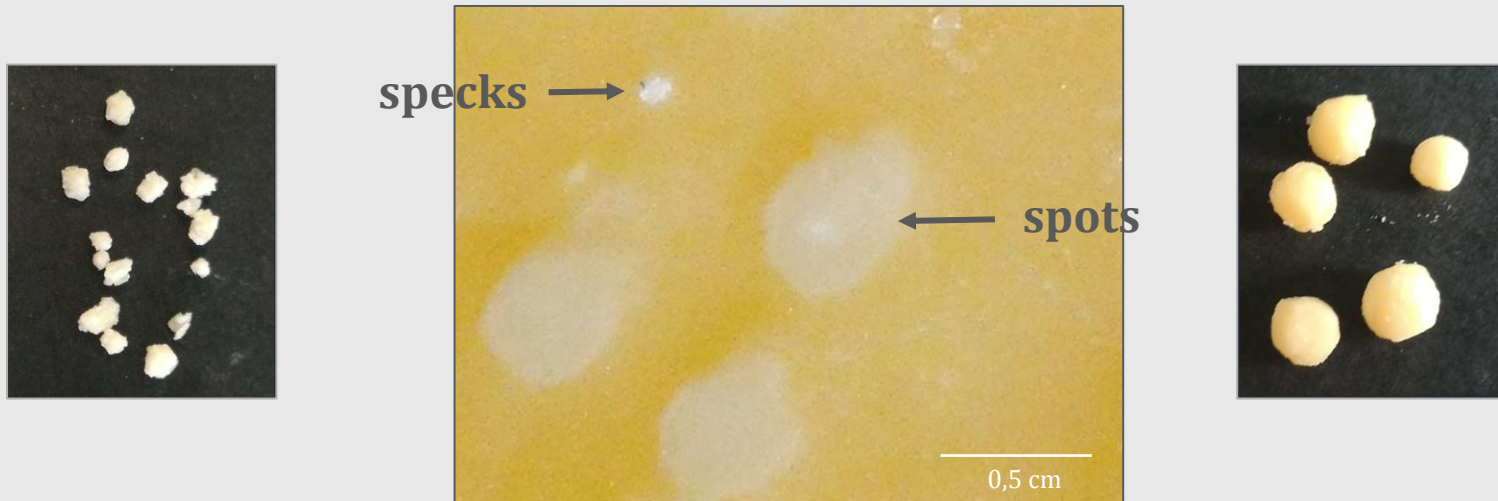
During ripening of cheese:

- Proteolysis of casein
- Humidity loss



Scope and nomenclature

To shed light on features and origins of **specks** and **spots** in extra hard cheeses



... **micro crystals** were also investigated

SPECK INVESTIGATION

- Light Microscopy

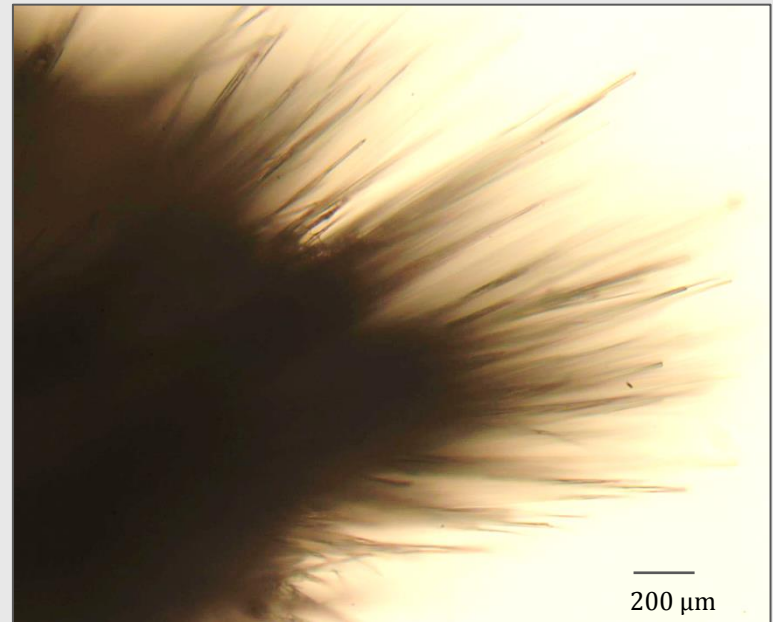
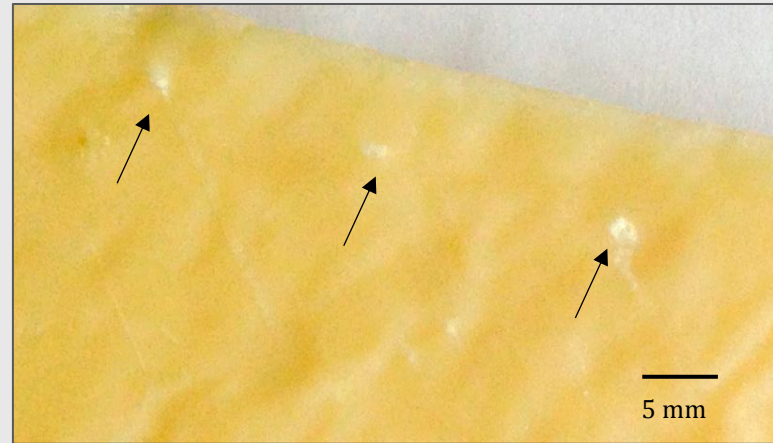


- Confocal Micro Raman



SPECKS: Light Microscopy

- **Numerous** inside and on cheese
- **Hard** and **White** against the darker cheese
- Needle-like **crystalline** substructure
- Smaller than 3 mm



SPECKS

- Light Microscopy

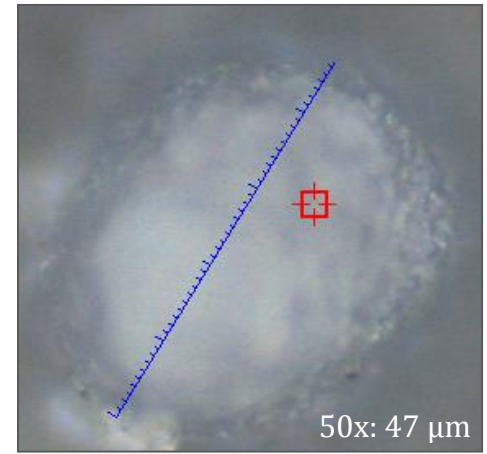
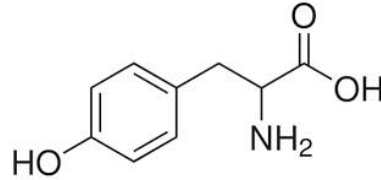


- Confocal Micro Raman



SPECKS

- SPECK FROM CHEESE
- PURE TYROSINE



94% matching with
pure **tyrosine**
from library

Raman Intensity (cps)

7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0

3000

2500

2000

1500

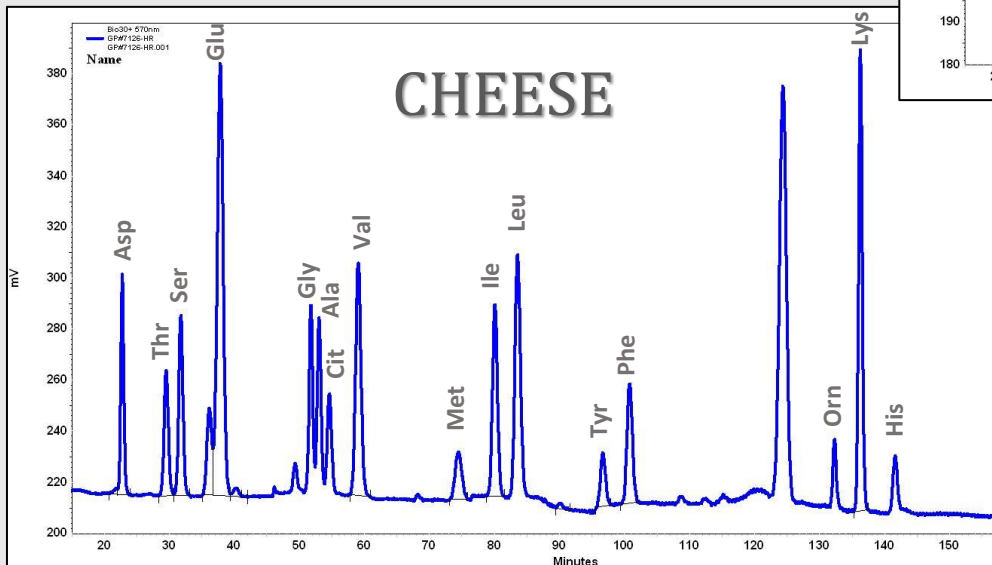
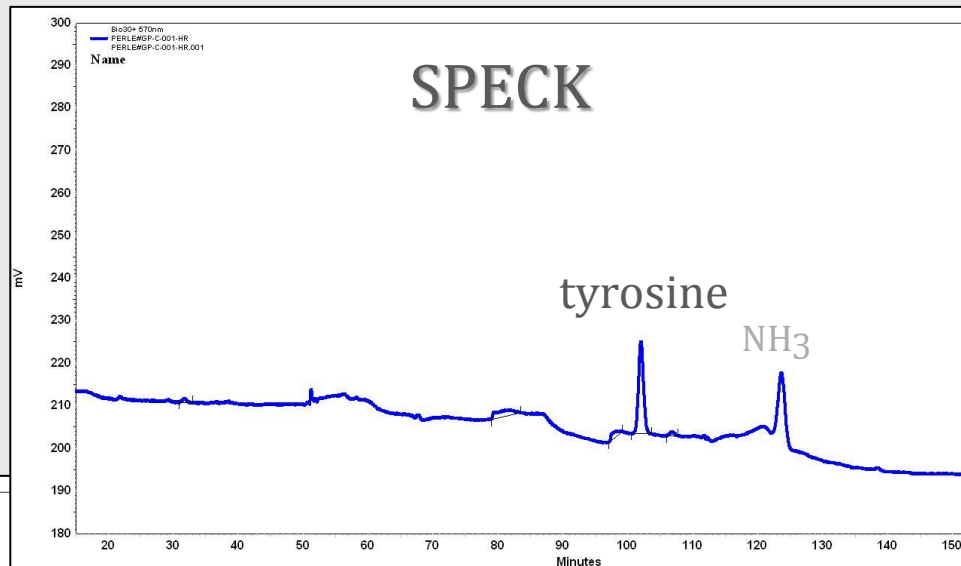
1000

500

Raman shift (cm-1)

SPECKS: CHEMICAL APPROACH

FAA composition was
determined







Result...

Speck is 85% **tyrosine**,
as reported by literature.

HYPOTHESIS OF THE GENESIS...

- Tyrosine has the lowest water solubility (0.45 mg/mL) among FAA
- During ripening of cheese, the water content decreases while the amount of free tyrosine increases
- In 12-month ripened cheese, the concentration of free tyrosine in water phase is around 6 mg/mL
- Increasing concentration of tyrosine → crystallization

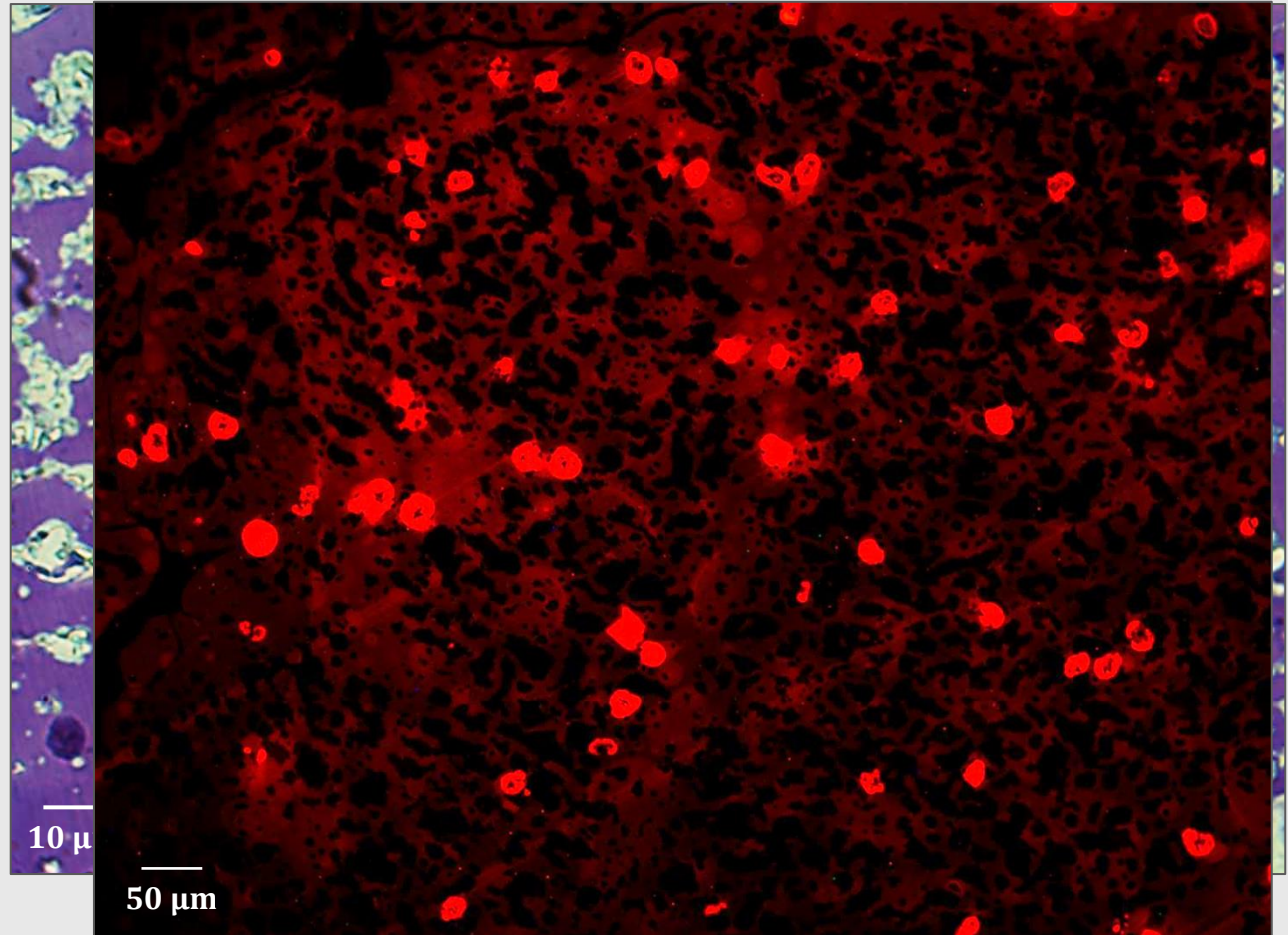
Microscopic crystals

- Light Microscopy 
- Confocal Microscopy 
- TEM 
- Confocal Micro Raman 


Microscopic crystals

- Light Microscopy: thin section

Crystal structures
are preferably
located in the
quantities 30-100g
of the junctions
between curd
particles



Microscopic crystals

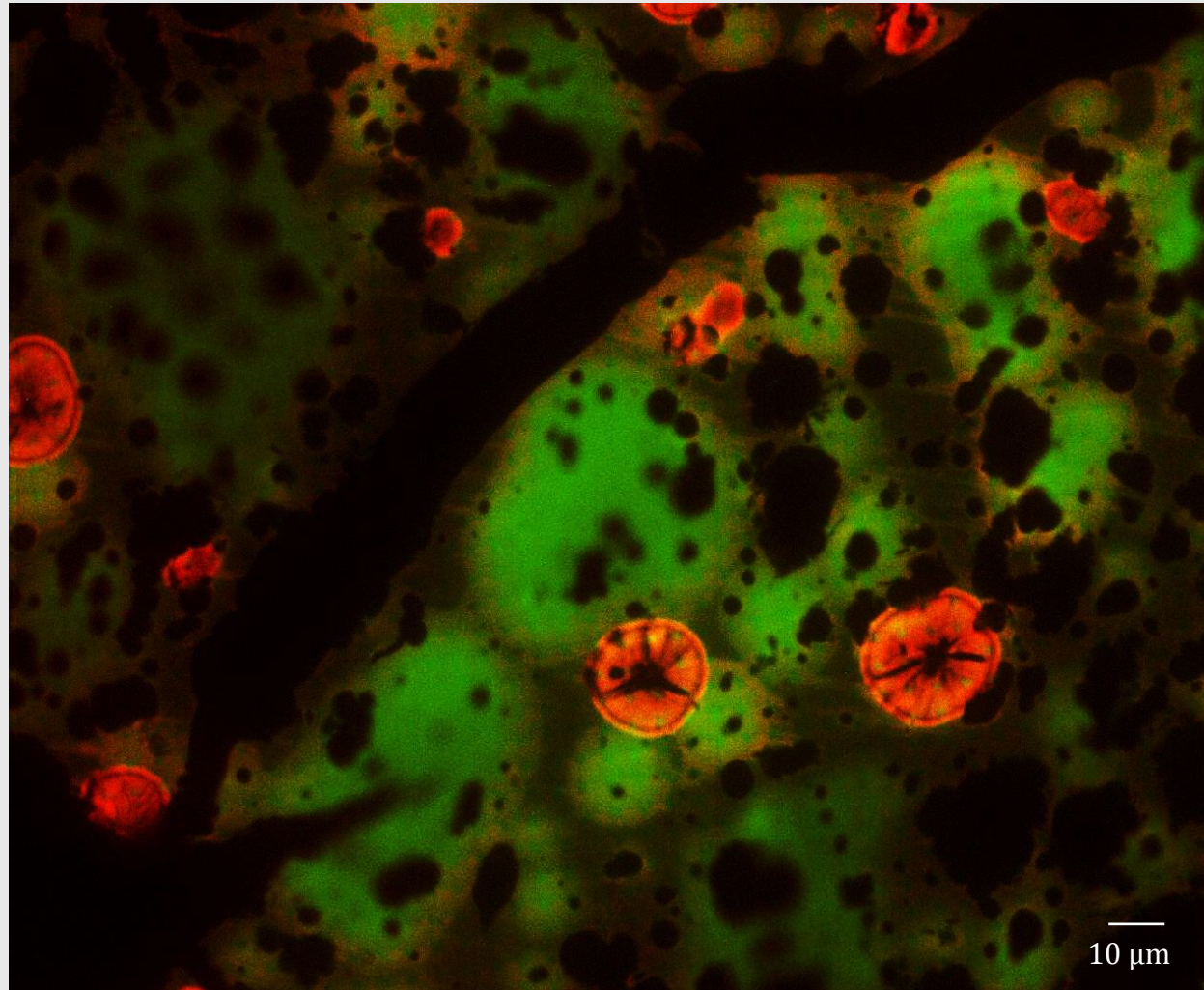
- Light Microscopy 
- Confocal Microscopy 
- TEM 
- Confocal Micro Raman 

Microscopic crystals



- Confocal Microscopy: thin section

Circular, oval or kidney-shaped structures

Sizes approximately range from 10-20 μm



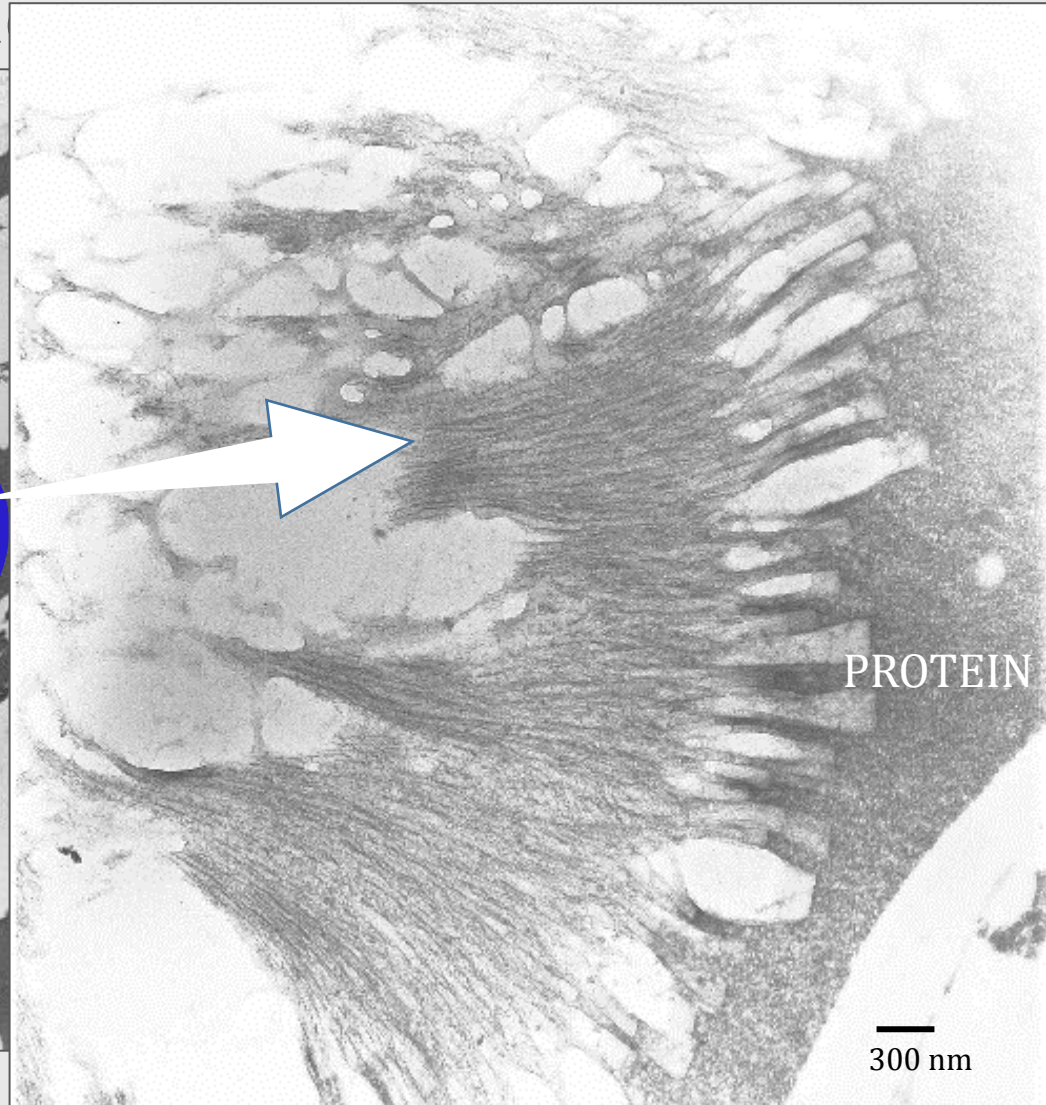
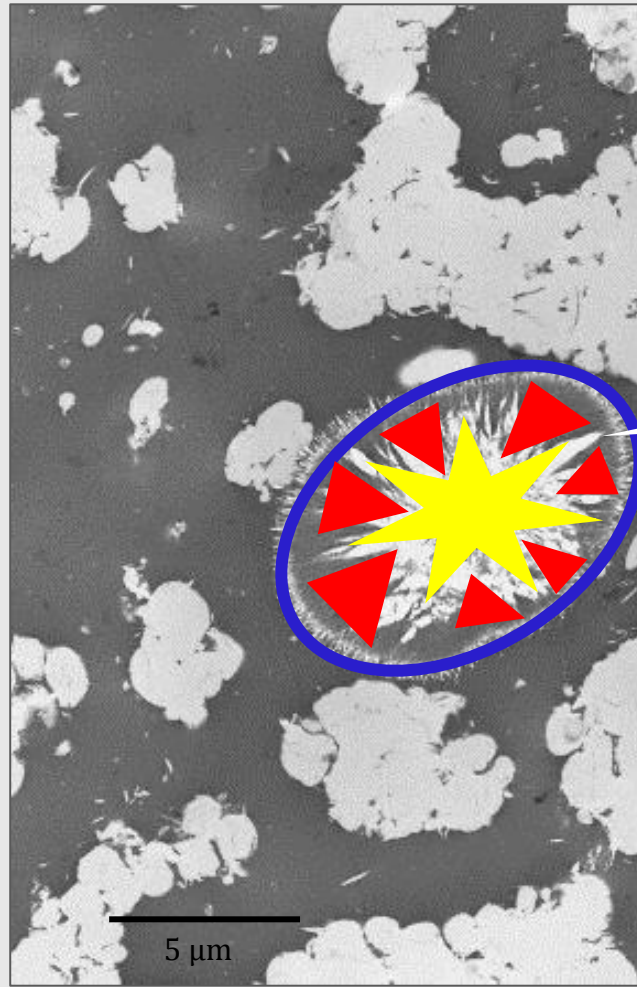
Microscopic crystals

- Light Microscopy 
- Confocal Microscopy 
- TEM 
- Raman 



Microscopic crystals

TEM: ultra thin section

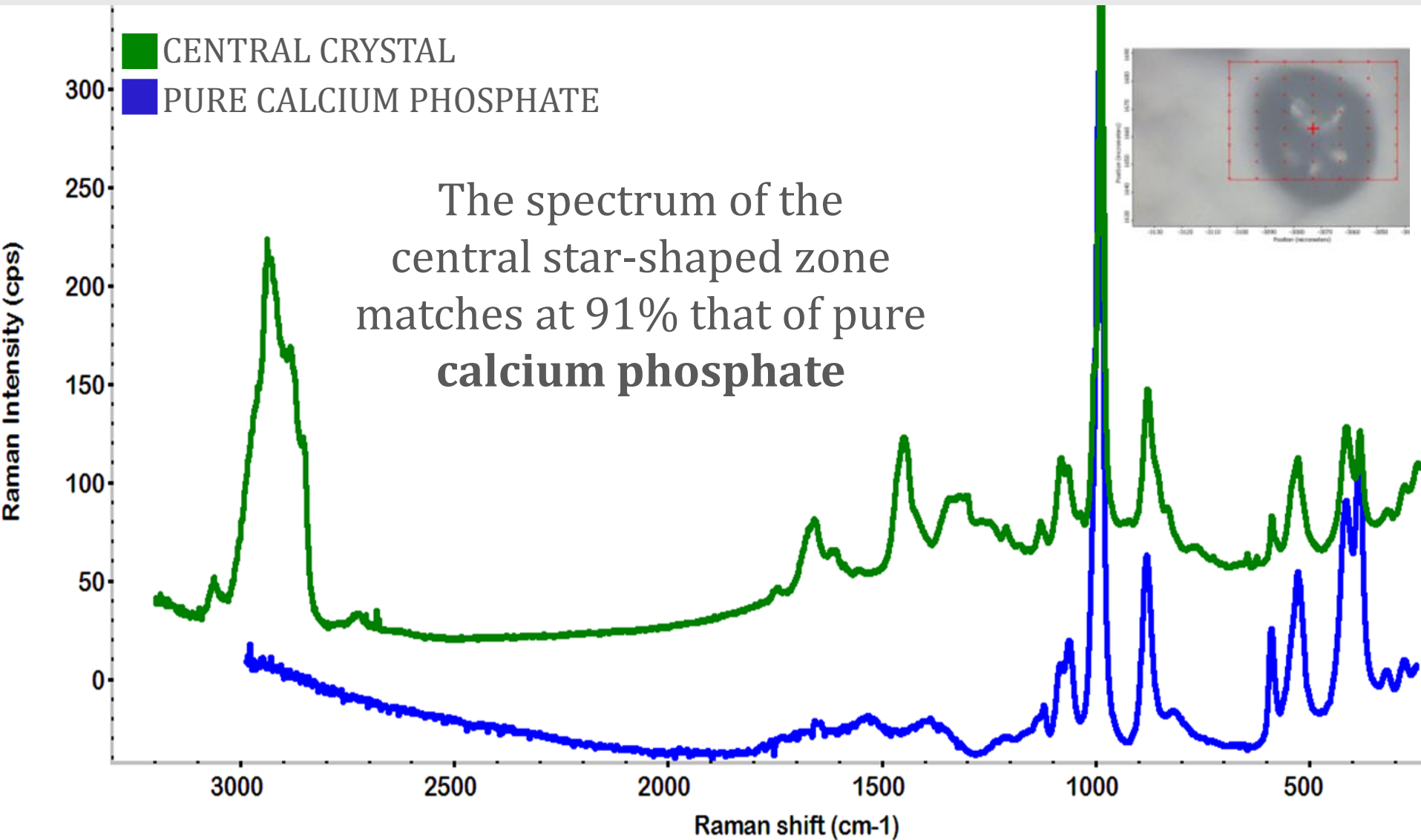


Microscopic crystals

- Light Microscopy 
- Confocal Microscopy 
- TEM 
- Confocal Micro Raman 



Microscopic crystals



HYPOTHESIS OF THE GENESIS...

- In fresh cheese, microbial lactic fermentation lowers the pH to 5-5,5 so that most of the calcium phosphate solubilizes from casein into the water phase
- During ripening, the water phase of the cheese progressively concentrates due to water loss



Crystallization of calcium phosphate



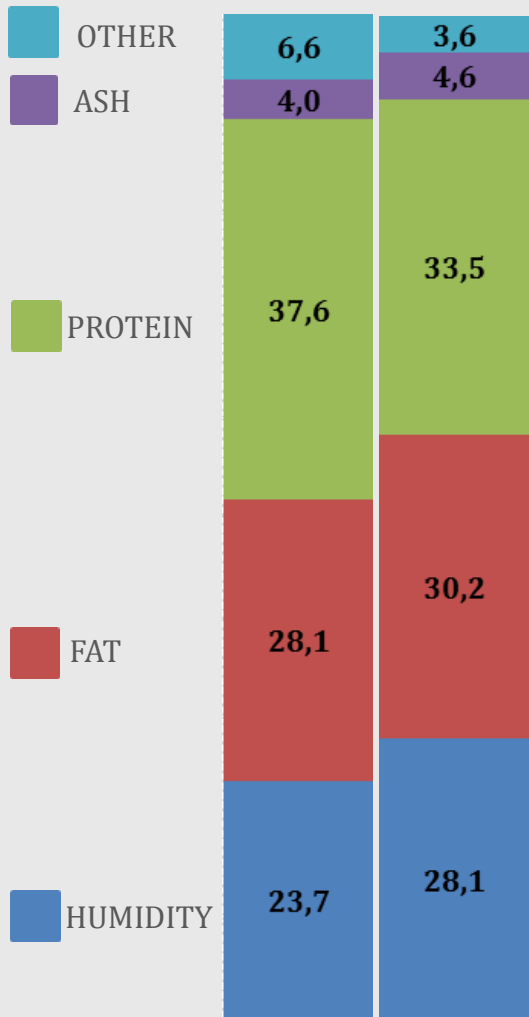
SPOT INVESTIGATION

- Spots are spherical, with diameter > 2 mm, located inside and on cheese slice
- Visible only after 1 year of ripening, more easily after 16-18 months
- Spots are made of white, soft, amorphous material and are easily collectible from the cheese
- In some cases they are so large and numerous that they may affect the texture and appearance of the cheese



SPOTS: CHEMICAL APPROACH

SPOT CHEESE

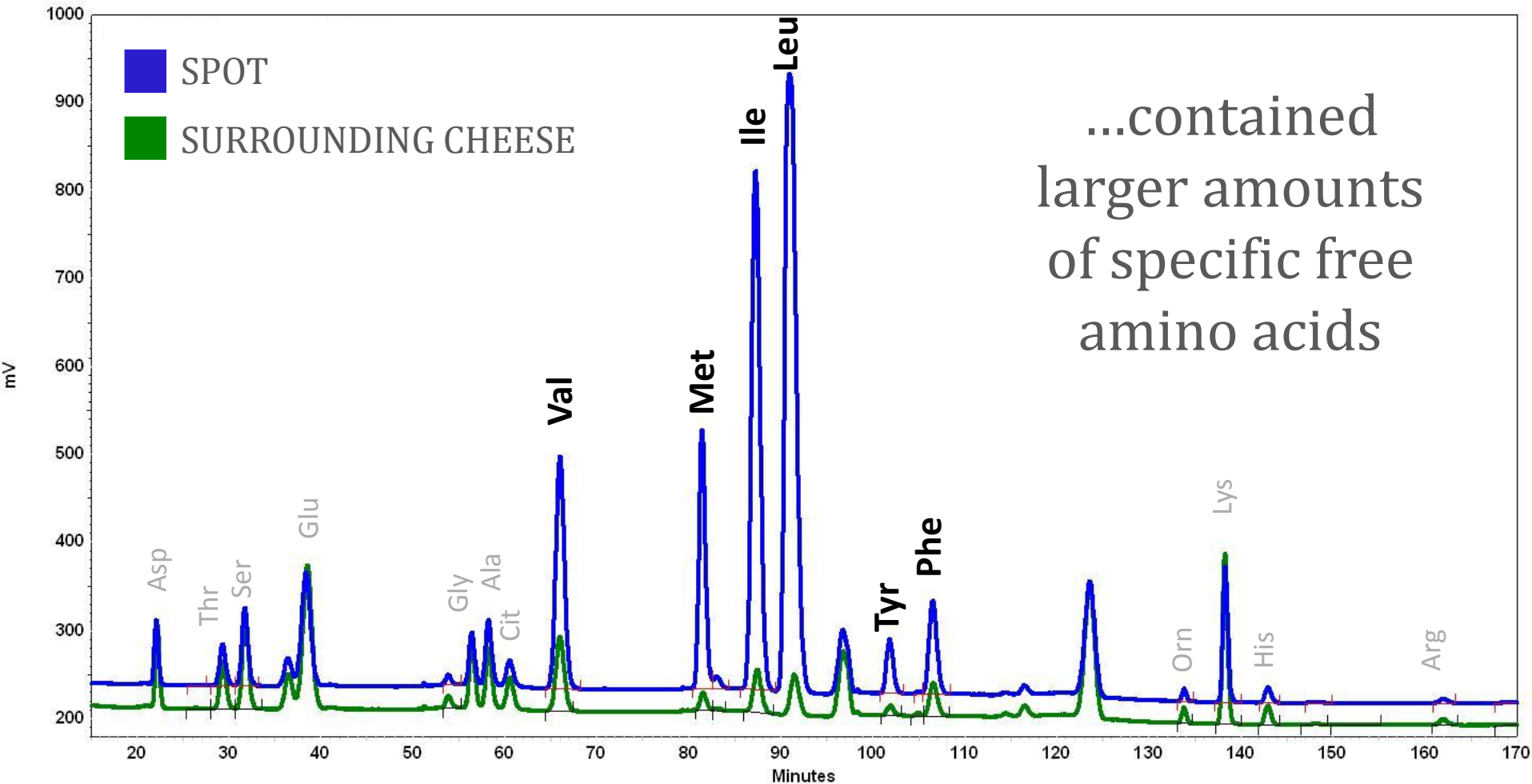


(g/100 cheese)



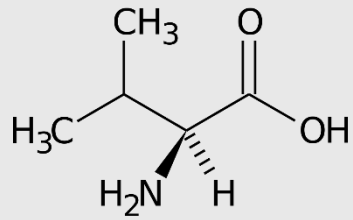
The SPOT is richer in protein and poorer in humidity, fat and ash

SPOTS: CHEMICAL APPROACH

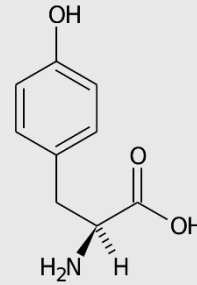


...contained
larger amounts
of specific free
amino acids

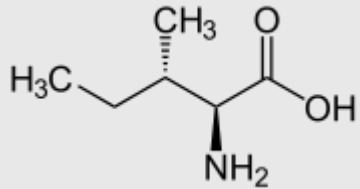
SPOT FAA



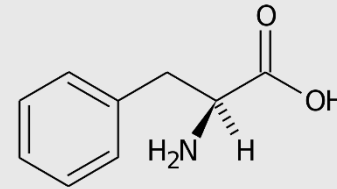
Valine



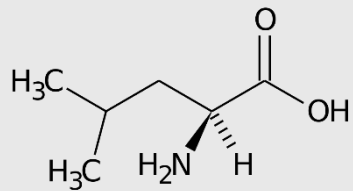
Tyrosine



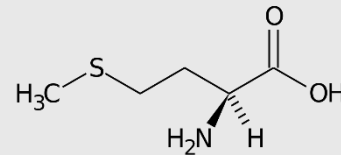
Isoleucine



Phenylalanine



Leucine



Methionine

Aromatic
ring

Branched
chain

Hydrophobic



SPOT INVESTIGATION

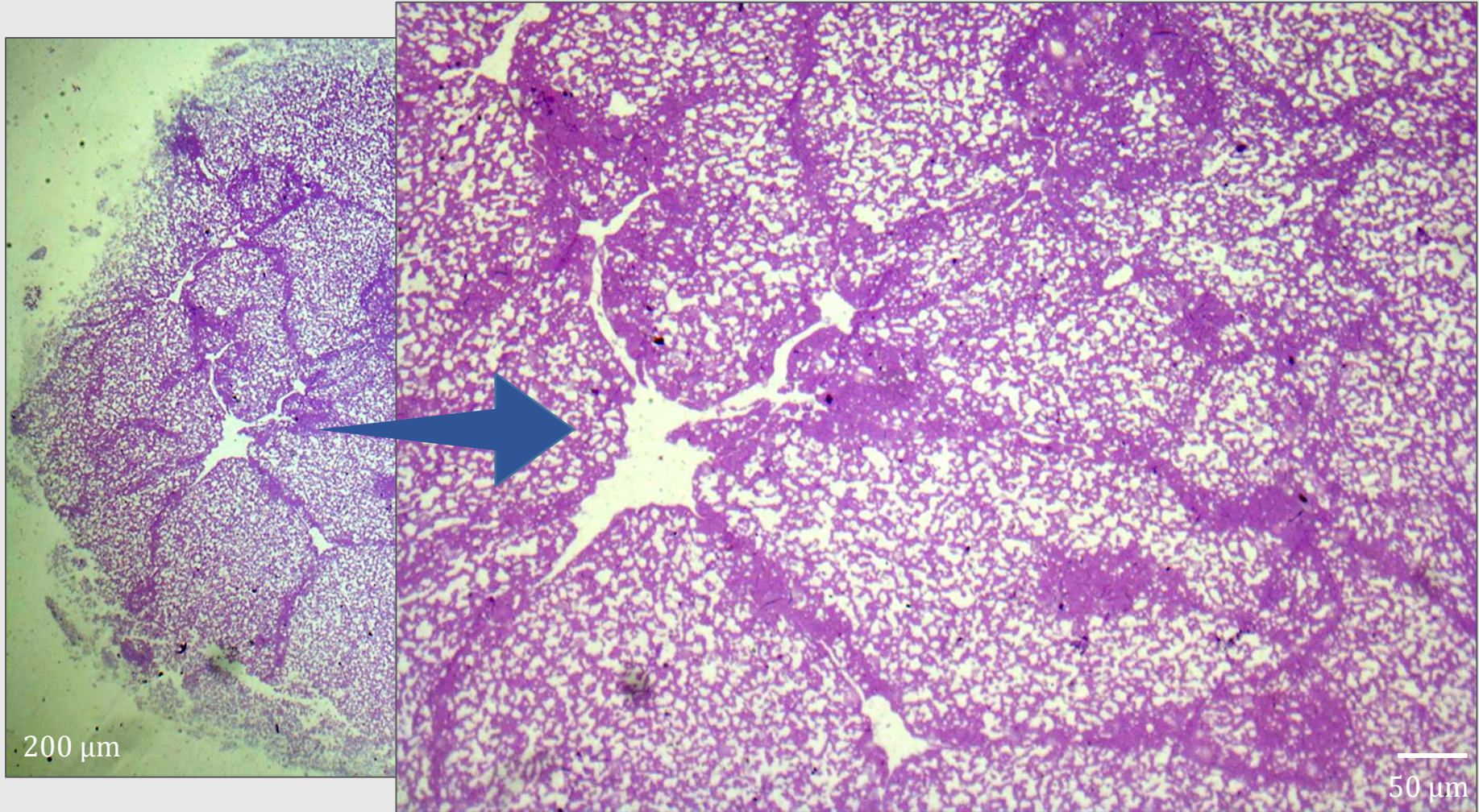
- Light Microscopy 

- TEM 



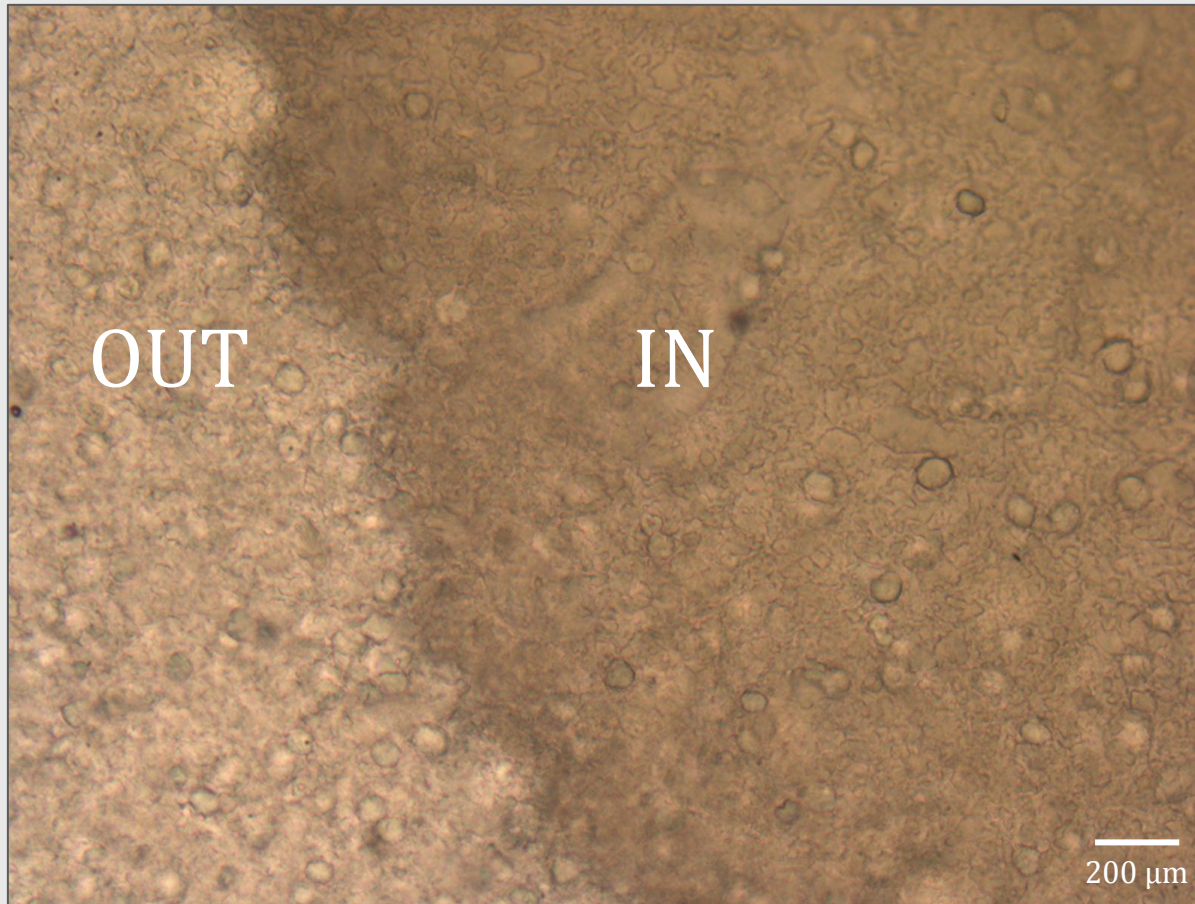
SPOTS

- Light Microscopy: thin section



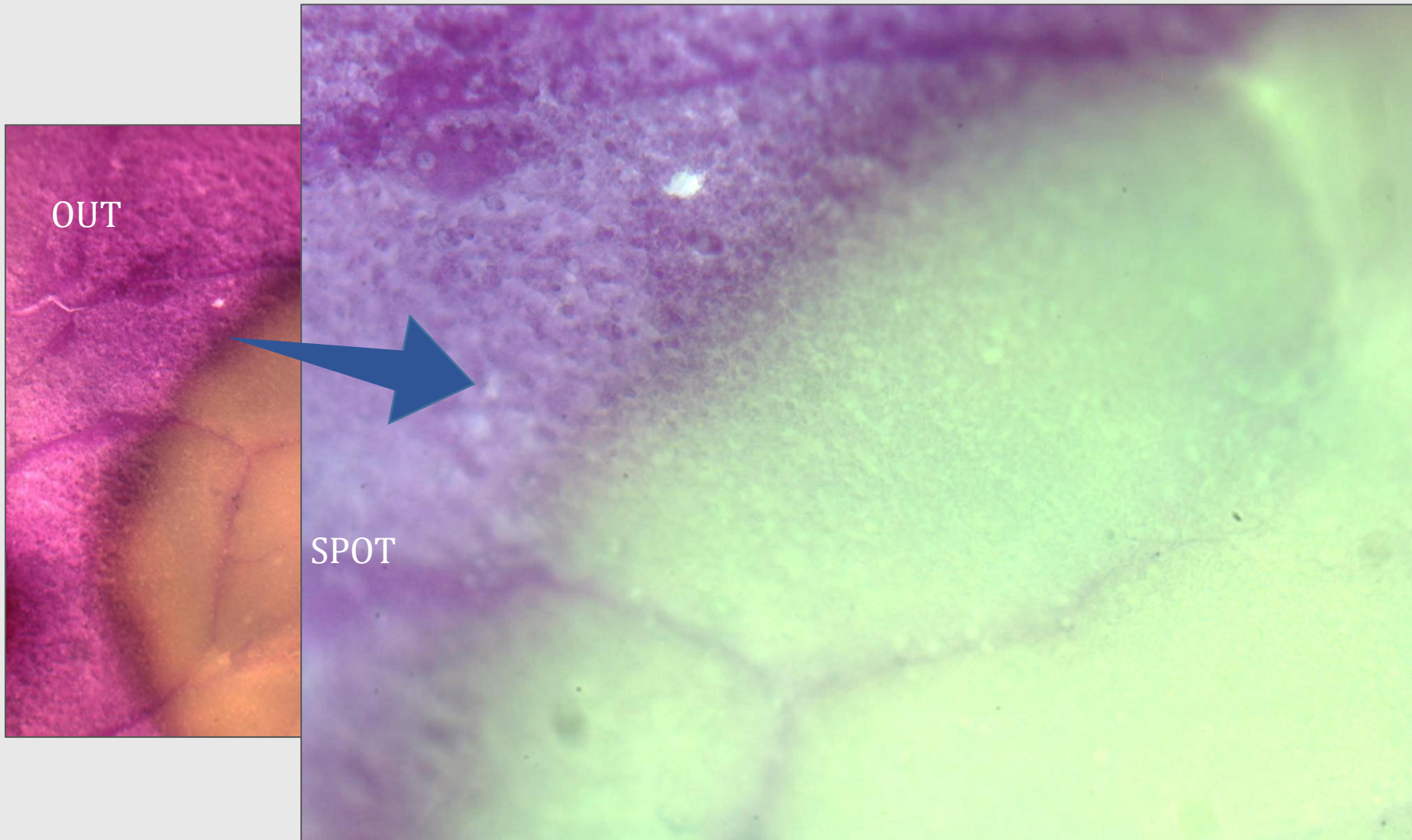
SPOTS

- Light Microscopy



The spot is denser than external cheese matrix

SPOTS IN NINHYDRIN



SPOTS

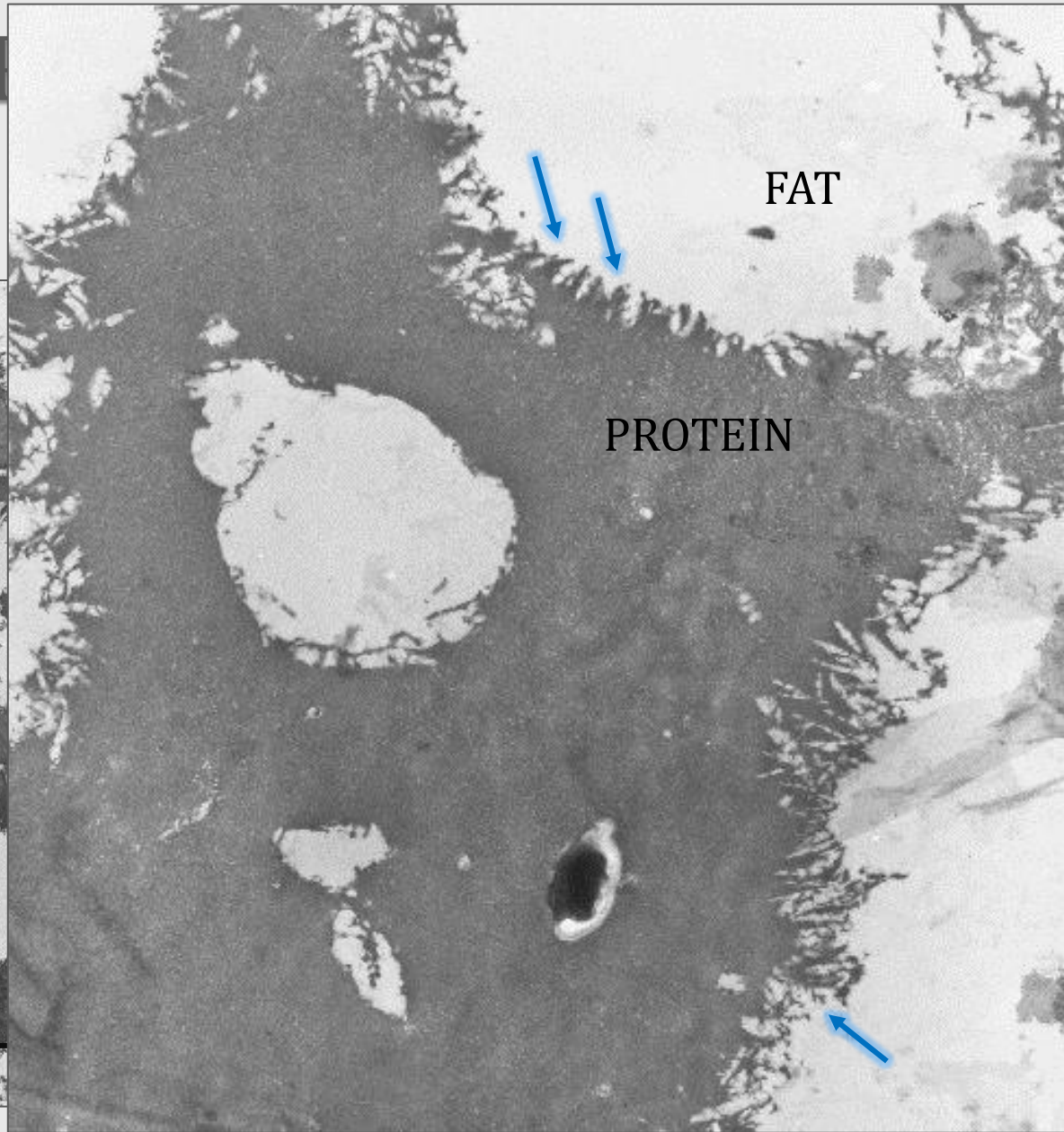
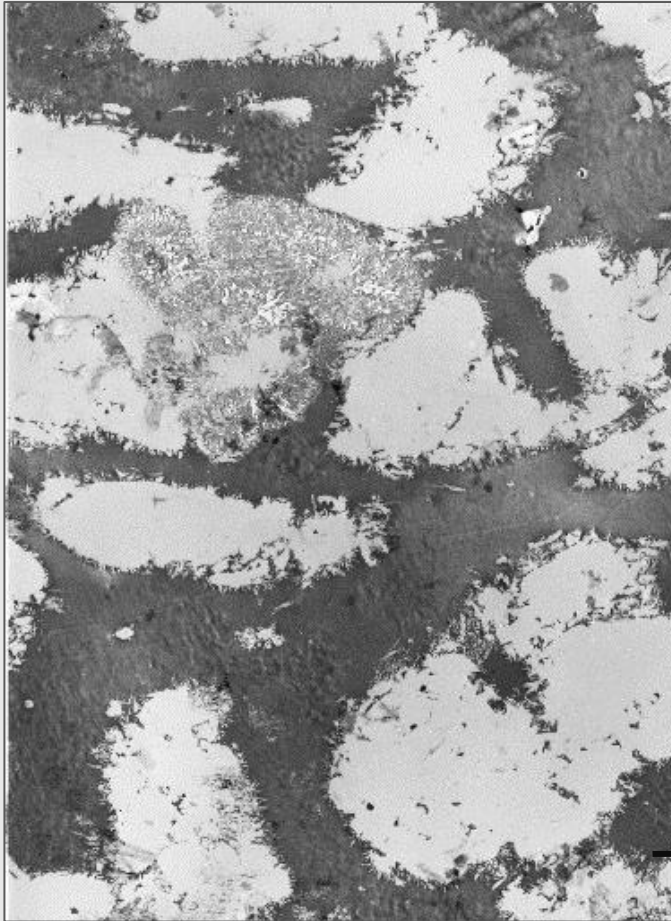
- Light Microscopy 

- TEM 



TEM: ULTRATHIN

SPOT



COMBINING THE INFORMATION...

RIPENING

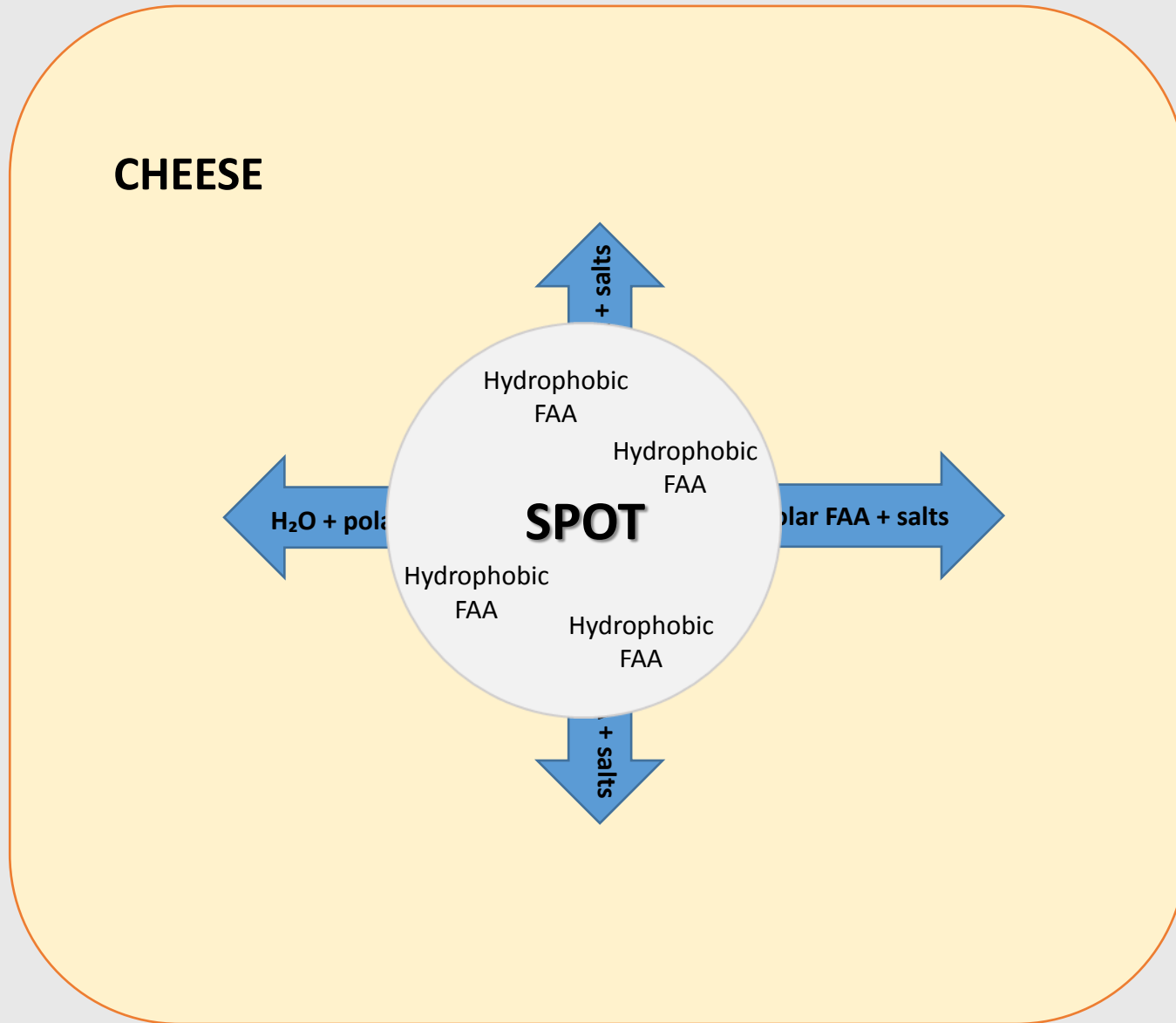
FAA ↑
HUMIDITY ↓

SPOTS

WITH CHEESE AGE ↑
HYDROPHOBIC FAA ↑
FIRMER SCAFFOLDING



HYPOTHESIS OF THE GENESIS...



CONCLUSIONS

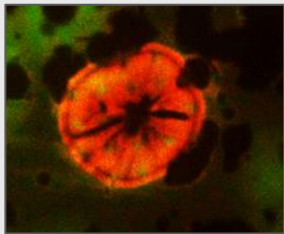
- Specks are crystals of **tyrosine**
- Microscopic crystals are **calcium phosphate** crystals
- Spots are amorphous concentrations of **hydrophobic FAA** such us **Leu, Ile, Val, Met, Phe** and **Tyr**
- Crystals and spots appear at different ripening times

Acidified curd

9-month ripened cheese

18-month ripened cheese

Time of ripening



CALCIUM PHOSPHATE CRYSTAL

TYROSINE CRYSTAL

SPOT

CONCLUSIONS

Because crystals and spots increase with the age of the cheese, their development must be interpreted as ripening phenomena and as a warranty of properly ripened cheese



A long, narrow aisle in a cheese cellar, lined with rows of wooden cheese wheels (Emmentaler) stacked on shelves. The perspective is from the end of the aisle, looking down its length. The ceiling is a curved, ribbed structure. The floor is a reddish-brown color. The text "Thank you for your kind attention" is overlaid in the center of the image.

Thank you
for your
kind
attention